Considerations for Evaluating Chemical Mutagenicity to Germinal Cells

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Modern technology has created a vast pool of chemical pollutants that invade man's environment daily. The mutagenic, teratogenic, and carcinogenic potentials of the majority of these chemicals have not yet been evaluated. It is vital to screen as many of these substances as is feasible for their genetic risks, with the ultimate goal of eliminating the potentially hazardous compounds from the environment, thereby reducing genetic damage in man.

In evaluating the genetic hazards of a chemical to man, consideration must be given to mutations caused to both somatic and germinal cells. In the case of mutations to somatic cells, the primary concern is about the potential of carcinogenesis—a concern to the immediate individual. For mutations to germinal cells, the risks are to the future population. No test system today is adequate to truly assess the risk to all types of somatic and germinal cells.

To evaluate mutagenic effects on germinal cells, three mammalian test systems are generally available. These are the dominant-lethal test (*1–4*), the specific-locus test (*4*, *5*), and the translocation test (*6*, *7*). Of these systems, the dominant lethal test has been more widely accepted and evaluated, partly because it is less expensive and simpler to perform.

In the dominant-lethal test, rodents are treated with the chemical agent and then mated with untreated partners. In general, the animal treated is a male because chemicals acting systemically on females may interfere with hormonal status, possibly interfering with the development of normal fetuses, or the chemical may act directly on the maturing oocyte, causing death other than by a dominant-lethal mutation (*3*). Furthermore, a mutational response in a male can be studied by mating it to more than one female at one or different times, an important point in evaluating the stage of spermatogenesis affected by the chemical, while the response in a female can only be studied in a single pregnancy (*3*). In most standard studies, males are treated by acute, subacute, or chronic exposures and then mated with untreated females. The females are replaced each week throughout a complete spermatogenic cycle to ensure testing of the animal at all stages of spermatogenesis. Mated females are sacrificed midway through the gestation period for examination of the uteri and for determination of the number of live and dead embryos. Deaths are assumed to result from a dominant lethal mutation.

The basis for using the dominant-lethal test is that nonviable zygotes can result from chromosomal damage and rearrangements. Evidence for this has been provided embryologically and cytogenetically (*6*, *8*). The primary advantages of using the dominant lethal test have been described (*3*, *6*, *9*) and are: (1) compared with other tests for meas-

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uring mutagenesis in mammalian germinal cells, it is relatively inexpensive; (2) it is possible to analyze mutational events in all mammalian germ cells in a reasonable time period; (3) treatment modes can be of all types—intraperitoneal, intramuscular, intravenous, oral, inhalation—and of acute, subacute, or chronic durations; (4) sensitivity of all stages of spermatogenesis and oogenesis can be evaluated.

Disadvantages have also been listed for the dominant lethal test. Some of them are: (1) nongenetic causes can also cause preimplantation loss (*3, 9*); (2) direct genetic analysis of mutation is not possible (*9*); (3) point mutagens will probably go undetected (*3, 7, 9*), a fact which is particularly sign ficant, since point mutations are more likely to be transmitted to the offspring than chromosome aberrations (*10*); (4) chromosome breakage—cause of dominant lethals—does not necessarily result in a mutation (*3, 9*); (5) significance of the results from treated females may be questionable, since fetal death may result from physiological rather than genetic effects. Therefore, effects on oogenesis may not be truly evaluated—an essential consideration (*7*).

In the specific locus test, wild-type male or female mice are treated with the test agent and then mated to partners that are homozygous for several recessive genes. The progeny are examined for the expression of a recessive gene, which can result only if one of the dominant genes undergoes a mutation. The phenotypic responses usually evaluated deal with coat color and morphological characteristics.

In the case of the specific locus test, the primary drawbacks that have limited its use are: The large number of animals and expense involved in performing the test and the relative insensitivity (*11*), presumably resulting primarily from the small number of genes being examined.

If these disadvantages can be overcome, there are many advantages to using the specific-locus test; it measures actual heritable responses, since the observed responses are transmitted to the progeny (*11*); the mutations can be either from point mutations or from major chromosomal aberrations (*12*); the background level of mutation is extremely low (*11*).

The translocation test is devised to measure sterility and heritable semisterility in F_1 progeny of rodents exposed to a test agent. This is accomplished by treating males or females with the test agent, mating them with untreated animals, selecting F_1 animals (usually males) for further breeding, and examining the litter size produced by the F_1 animals. In general, any animal showing greater than 50% fetal death is considered semisterile. An animal considered to contain heritable semisterility can be mated further for additional data, and the germinal cells can be examined cytogenetically to confirm that chromosomal translocations are present. The semisterility has been shown to be the result of heritable translocations that can be detected cytogenetically.

The primary disadvantage of the translocation test is the length of time required to perform the test (approximately 6 months) and the expense involved. However, the advantages may prove to compensate for these drawbacks. The primary advantages are that it is truly a measure of a heritable response (*13*); an animal suspected of being a mutant can be examined cytogenetically to confirm that translocations are present (*13–15*); the background level is extremely low, while the positive responses can be quite high (*13*).

Various modifications could decrease the complexity of the translocation test, so that it will not be a great deal more expensive than the dominant lethal test without compromising the quality of the test.

The current philosophy appears to be that the dominant lethal test should be used in a general screening program. However, since the sensitivity of the dominant-lethal test (as well as the translocation and specific-locus test) is much less than with simpler organisms (*9, 10*), it seems logical that these tests be relegated to a second phase in the evaluation of chemical agents—that is, to further evaluate compounds that have been shown to have an effect on the genome in a prescreen system having the greatest level of sensitivity. If the function of mammalian test system i.e., to evaluate the genetic effects of a chemical on germinal cells, were a secondary rather than a primary screen, the additional costs of the specific locus or translocation tests would not be as significant.

A general mutagenic evaluation system that takes all these points into consideration might be as follows: (1) a decision process to determine which compounds should be tested; (2) a presumptive screen to establish further testing priorities, and (3) extensive mammalian testing.

A decision process to determine which compounds should be tested would evaluate (*8, 10*): the extent of human exposure, a knowledge of the genetic effect of the agent, structural relationship to known mutagens, and the potential of any metabolites causing mutagenesis.

In presumptive screening to establish further testing priorities, the most sensitive system available would be used to determine whether there is a potential for an effect on the genome. Potential for metabolic activation should be used. The systems to be used could include the host-mediated assay with microbial cells (*16, 17*), the host-mediated assay with mammalian cells (*18*), mammalian cells in tissue culture combined with a metabolic activation system (*19*), and *in vitro* microbial studies with the use of a metabolic activation system (*20, 21*).

Extensive mammalian testing, including a translocation or a specific-locus test would provide as much information as possible for extrapolation to man. The route of administration of the test material would be done on a chronic basis. In addition to tests of germinal cell effects, studies on somatic cells should also be performed (e.g., tissue culture and cytogenetic studies).

Because of the limitations described for the dominant lethal test, it certainly appears that the specific locus test or the translocation test would be more meaningful when discussing relevance to man. However, the need remains to conduct definitive studies on these tests to establish levels of sensitivity and correlations with other test systems. Studies such as those described by Ehling (*11*), where comparative results between the specific-locus test and the dominant-lethal test were made, and those performed by Generoso (*13*) on the translocation test must be performed. In Ehling's (*11*) studies, a comparison was made between methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), isopropyl methanesulfonate (iPMS), *n*-propyl methanesulfonate, triethylenemelamine (TEM), and 2-methoxy-6-chloro-9-[3-ethyl-2-chloroethyl) amino propylamino]acridine dihydrochloride (ICR–170). In general, he found a lower level of sensitivity in the specific locus test than in the dominant lethal test. Generoso (*13*) showed that the translocation test was more sensitive in detecting an adverse effect for EMS than the dominant-lethal test. A dose of 150 mg/kg was required to induce a detectable response in the dominant-lethal test, while 50 mg/kg was positive in the translocation test. Also of extreme significance was the fact that in the negative controls, no translocations were found in the 1102 F_1 males tested, while 98 F_1 males treated with 200 mg/kg of EMS 33 showed positive responses. This indicates, therefore, that extremely low background frequencies exist and that significantly high positive responses occur. Definitive studies such as these two must be conducted. In these studies, consideration should also be given to routes of administration more relevant to man, such as the oral route or inhalation, and to the pharmacological question of whether the compounds actually reach the sites being tested—in this case, the germinal cells.

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